Topical Review

Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals?

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The incidence of the metabolic syndrome, a cluster of abnormalities focusing on insulin resistance and associated with high risk for cardiovascular disease and diabetes, is reaching epidemic proportions. Prevalent in both developed and developing countries, the metabolic syndrome has largely been attributed to altered dietary and lifestyle factors that favour the development of central obesity. However, population-based studies have suggested that predisposition to the metabolic syndrome may be acquired very early in development through inappropriate fetal or neonatal nutrition. Further evidence for developmental programming of the metabolic syndrome has now been suggested by animal studies in which the fetal environment has been manipulated through altered maternal dietary intake or modification of uterine artery blood flow. This review examines these studies and assesses whether the metabolic syndrome can be reliably induced by the interventions made. The validity of the different species, diets, feeding regimes and end-point measures used is also discussed.

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Introduction

The metabolic syndrome, associated with the rising incidence of obesity in developed countries, is reaching epidemic proportions, affecting between 24% and 34% of the US population (Wilson & Grundy, 2003) and up to 36% of Europeans aged 40–55 (Balkau *et al.* 2002). The syndrome, which may progress to type 2 diabetes mellitus and cardiovascular disease, is classically defined as a combination of three of the following five disorders: raised blood pressure, central adiposity, raised serum triglycerides, lowered serum HDL cholesterol and fasting hyperglycaemia (National Health and Nutrition Survey III, discussed in Wilson & Grundy, 2003). Table 1 summarizes the current clinical diagnostic criteria and relevant methods of measurement. Inevitably, as the metabolic syndrome is better classified and understood, new criteria are included. In a recent review, Reilly & Rader, 2003) include diagnostic indices of inflammation, oxidative stress and prothrombotic factors as biomarkers of the metabolic syndrome. Others have suggested inclusion of leptin resistance (Arch *et al.* 1998) and endothelial cell activation (Bonora *et al.* 2003) and highlighted the possibility that endothelial dysfunction may not be one of the sequelae of the metabolic disorder, but a primary facet of this syndrome.

The 'fetal origins' hypothesis

A substantial body of epidemiological evidence now suggests that a poor *in utero* environment elicited by maternal dietary or placental insufficiency may 'programme' susceptibility in the fetus to later development of cardiovascular and metabolic disease. Most of these investigations were stimulated by the 'fetal origins' hypothesis proposed by Barker and colleagues, which associated adulthood hypertension, insulin resistance and dyslipidaemia to adverse intra uterine conditions in middle to late gestation which lead to disproportionate fetal growth (Barker, 1995, 1997; Godfrey *et al.* 1997). This hypothesis evolved as a result of further investigation, and 'programming' is now more commonly ascribed to any situation where a stimulus or an insult during development establishes a permanent physiological response (Lucas, 1991). The suggestion

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Table 1. Clinical disorders that contribute to the metabolic syndrome and methods of measurement

Disorder	Method/parameter measured	Reference
Obesity	DEXA scan, body mass index, lean muscle mass, bodyweight	Bonora *et al.* (2003), Reilly & Rader (2003)
Insulin resistance	Euglycaemic/hyperinsulinaemic clamp, fasting plasma insulin and glucose concentrations, oral glucose tolerance test	Bonora *et al.* (2003), Reilly & Rader (2003)
Hypertension	Sphygmomanometry, automated plethysmography	Bonora *et al.* (2003), Reilly & Rader (2003)
Dyslipidaemia	Plasma LDL:HDL cholesterol ratios, fasting plasma triglyceride concentrations, cholesterol flux	Bonora *et al.* (2003), Reilly & Rader (2003)
Inflammatory response	Plasma cytokine concentration, serum soluble cell adhesion molecule concentrations, plasma C-Reactive Protein concentrations	Riserus *et al.* 2002, Bonora *et al.* (2003), Reilly & Rader (2003)
Oxidative status	Plasma isoprostane concentrations, concentration of plasma antibodies to oxidized LDL cholesterol, superoxide generation and scavenging capacity (erythrocyte glutathione, superoxide dismutase, catalase) activity)	Riserus *et al.* 2002, Bonora *et al.* (2003)
Leptin insensitivity	Plasma leptin:body fat ratios, plasma leptin concentrations	Arch *et al.* (1998), Bonora *et al.* (2003)
Endothelial dysfunction	Brachial artery plethysmography, reactive hyperaemia	Bonora *et al.* (2003)

Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein.

that the intrauterine environment may have permanent effects on the fetus is far from novel, although the concept that developmental programming may contribute substantially to adulthood diseases has led to a revival of interest in the influence of the *in utero* environment on the fetus and neonate.

The 'thrifty phenotype hypothesis' proposed by Hales & Barker (1992) attempts to explain the associations between low birthweight and adult type 2 diabetes. In survival terms, this hypothesis is plausible. If *in utero* nutrition is poor, then predictive adaptive responses are made by the fetus to maximize uptake and conservation of any nutrients available, resulting in a conservative metabolism. When the infant is then exposed to a similarly deficient postnatal diet to that experienced *in utero*, the programming of the thrifty phenotype will confer a 'predictive adaptive' advantage, as these individuals would then be biologically prepared to withstand the poor diet. The problems arise, however, where postnatal diet is adequate or plentiful and exceeds the range of the predictive adaptive responses. The consequence of this, it is proposed, is development of the metabolic syndrome. There is currently little understanding of the exact range of tolerance of predictive adaptive responses, but it follows that disease becomes manifest when the postnatal nutrient environment is considerably different from that predicted from the *in utero* experience (Gluckman & Hanson, 2004).

Developmental programming of the metabolic syndrome: epidemiological evidence

The earliest epidemiological studies of fetal programming focused on the relationship between birthweight and adult disease in geographically localized populations. Barker and colleagues showed a relationship between birthweight and adult disease (see Barker *et al.* 1989, 1990, 1993*a*), including facets of the metabolic syndrome. Thus birthweight has been linked to adulthood hypertension (Barker *et al.* 1990), insulin resistance (Phillips *et al.* 1994), vascular dysfunction (Martyn *et al.* 1995), obesity (Yajnik, 2000) and dyslipidaemia (Barker *et al.* 1993*b*). The importance of maternal nutrition, and the effect that poor nutrition may have on birthweight and subsequent adulthood disease was addressed in studies of exposure to famine, most notably the Dutch Hunger Winter (Ravelli *et al.* 1976, 1999; Roseboom *et al.* 1999, 2001*a*).

Exposure to the Dutch famine (where average daily caloric intake was limited to 1680–3360 kJ) during late gestation was associated with increased adult obesity and glucose intolerance, and famine exposure early in gestation resulted in hypertension (Ravelli *et al.* 1976, 1998). Furthermore, smaller babies (particularly females) faced a higher risk of adult onset diabetes (Roseboom *et al.* 2001*a*). A range of studies in disadvantaged populations in the USA, South Africa (Levitt *et al.* 1999), the Caribbean (Thame *et al.* 2000), India (Fall *et al.* 1998) and Australia (Hoy *et al.* 1999) have identified cardiovascular risk to be greater in populations suffering from poor *in utero* nutrition. However, the strength of the relationship between birthweight and later cardiovascular disease has been challenged (Huxley *et al.* 2002). Some studies have shown no relationship between maternal diet and adult disease, for example data from individuals conceived during the siege of Leningrad (where caloric intake was reduced) did not show any association of birthweight with adult glucose homeostasis (Stanner *et al.* 1997). The predictive adaptive response hypothesis may provide an explanation for this observation, as nutritional status both before and after the famine periods in Leningrad was

poor, and predictive adaptive responses may therefore have been appropriate for the postnatal environment experienced. Nutrition following the Dutch famine was, however, relatively plentiful and the subsequent adult nutritional plane was higher than that predicted by the predictive adaptive responses and therefore disease was manifest.

In accord with the theory of predictive adaptive responses, data from other population-based studies (Primatesta *et al.* 2003) may suggest that growth restriction *in utero* is less harmful to long-term health when nutrition in postnatal life is compromised to a similar degree. Conversely, and in keeping with the hypothesis, 'catch-up growth', where babies are born small (lower percentiles of the z distribution), but grow rapidly in the first months of life to eventually achieve normal centiles, has been identified as a particular risk factor for the later development of cardiovascular disease, and/or the metabolic syndrome (Eriksson *et al.* 1999).

The animal models

In view of the shorter life span of animals and because genetic and environmental influences can be carefully controlled in animals, a substantial effort in recent years has centred upon the establishment of animal models of developmental programming which may have relevance to the human condition. Remarkably, results from many of these investigations seem to suggest a common offspring phenotype with shared similarities of human metabolic syndrome. However analogies are often drawn without due attention to the specific criteria put forward in the clinical description of the disease (Table 1). In this review, we shall examine the literature for evidence that some, or the entire constellation, of the disorders which together define the metabolic syndrome may be 'developmentally programmed' in adult offspring subjected to placental insufficiency or to suboptimal maternal nutrition. Firstly, the various models will be summarized.

Placental insufficiency and hypoxia. Animal models of placental insufficiency may be induced by uterine artery ligation (Brown & Vannucci, 1978; Houdijk *et al.* 2000; Sanders *et al.* 2004*a*,*b*), uterine and umbilical artery embolism (Block *et al.* 1990; Gagnon *et al.* 1997; Bloomfield *et al.* 2002; Louey *et al.* 2003) or carunclectomy (Jones *et al.* 1988; Rees *et al.* 1998; Butler *et al.* 2002). Hypobaric hypoxia has also been used as a stimulus to produce intrauterine growth restriction (IUGR) and cardiovascular complications in offspring. For this purpose the sheep is an excellent species, as the fetus can be chronically instrumented; however, due to financial and logistical restraint most ovine studies describe fetal responses rather than adult phenotype (Jacobs *et al.* 1988; Fletcher *et al.* 2000, Fletchert *et al.* 2000; Gardner *et al.* 2001, 2002, 2003; Giussani *et al.* 2001; Llanos *et al.* 2002; Min *et al.* 2002). The placental insufficiency models are highly relevant to human pregnancy, particularly in developed countries where the majority of fetal growth restriction is attributed to placental disease. However, reduced maternal and fetal blood flow are accompanied by varying amounts of both hypoxaemia and nutrient restriction, factors that cannot be isolated one from the other. Additionally, the effect of hypobaric hypoxia on the utero-placental circulation varies between species; in guinea-pigs uterine artery flow is decreased, whereas uterine artery blood flow increases in ovines (White & Zhang, 2003). The degree to which these two key factors may operate as causative mechanisms of intrauterine growth restriction is also likely to be variable. As this review focuses on nutritional factors acting as stimuli to programming of metabolic syndrome, we will not discuss the role of placental insufficiency or hypoxia further, but acknowledge their importance as models of IUGR.

Dietary manipulation. Dietary manipulation by global caloric restriction, reduction of dietary protein content, iron restriction or dietary fat supplementation has been the focus of a large body of work, in both rodent and ovine models. By far, the most commonly described models of nutritional imbalance are those in which pregnant rats are subjected to malnutrition. The single largest limitation of the rodent models lies in fundamental differences between rat and human development. The rat is an altricial animal, born with a poorly developed CNS and autocrine system, and significant maturation occurs during the weaning period. The most obvious animals to circumvent this limitation are primates, but expense, housing requirements, lifespan and ethical considerations result in primates seldom being studied. The guinea-pig may be a more relevant species, as these animals are precocial, born with a well-developed CNS, endocrine and cardiovascular system, but surprisingly, perhaps because of cost, only a few studies have been undertaken. Because of the polyocous nature of pregnancy in both rats and guinea-pigs, the possibility arises of variable fetal and neonatal nutrient supply to individual offspring within the same litter. In contrast, sheep pregnancies are usually singleton or twin. Careful choice of sheep breed, maternal age and pre- pregnancy nutrition and body condition can be used as critical tools to direct the study towards key questions in human pregnancy.

Models of maternal dietary imbalance

Following the studies of subjects exposed in early life to the Dutch Hunger Winter (Ravelli *et al.* 1976, 1998, 1999; Roseboom *et al.* 1999, 2000*a*,*b*, 2001*a*,*b*), a number of animal models of maternal nutrient restriction were developed to mimic the caloric restriction experienced by pregnant women at that time. These have predominantly

Table 2. Composition of commonly used low protein diets, and respective control diets

Diet	Protein	Fibre	Starch	Simple sugars	Lipid	Vitamins	Minerals	Methionine	Reference
LP6%	Casein 6%	6.7%	15.3%	Sucrose 51.2%	Corn oil 15.0%	(AIN-76) 1%	(AIN-76) 0.35%	0.37%	Galler & Tonkiss (1991)
C25%	Casein 25%	5%	11.4%	Sucrose 38.2%	Corn oil 15.2%	(AIN-76) 1%	(AIN-76) 0.35%	0.37%	Galler & Tonkiss (1991)
SH 12%	Casein 12%	5%	46.5%	Sucrose 23.4%	Corn oil 10%	(AIN-76) 2%	(AIN-76) 0.5%	0.5%	Langley & Jackson (1994)
SH 9%	Casein 9%	5%	48.5%	Sucrose 24.3%	Corn oil 10%	(AIN-76) 2%	(AIN-76) 0.5%	0.5%	Langley & Jackson (1994)
SH 6%	Casein 6%	5%	50.5%	Sucrose 25.3%	Corn oil 10%	(AIN-76) 2%	(AIN-76) 0.5%	0.5%	Langley & Jackson (1994)
SHC 18%	Casein 18%	5%	42.5%	Sucrose 21.3%	Corn oil 10%	(AIN-76)2%	(AIN-76) 0.5%	0.5%	Langley & Jackson (1994)
LP9%	Casein 9%	4.3%	43.7%	Sucrose 27.1%	Corn oil 5%, lard 5%	(RP) 2%	(RP) 1%	0.15%	Woods *et al.* (2001)
C22%	Casein 21%	4.3%	43.7%	Sucrose 15.0%	Corn oil 5%, lard 5%	(RP) 2%	(RP) 1%	0.15%	Woods *et al.* (2001)
HF 9%	Casein 9%	5%	8%	Glucose 66.7%	Soy oil 4.3%	∼(AIN-76)*	∼(AIN-76)*	0.2%	Snoeck *et al.* (1990)
HFC 22%	Casein 22%	5%	8%	Glucose 53.7%	Soy oil 4.3%	∼(AIN-76)*	∼(AIN-76)*	0.2%	Snoeck *et al.* (1990)
LP 8%	Crude protein 8%	5.4%	65%	Sucrose 4.4%	Soy oil 3.3%	∼(AIN-76)*	∼(AIN-76)*	0.3%	Athauda *et al.* (2004)
C 22%	Crudeprotein 22%	15%	33%	Sucrose 5.1%	Soy oil 4.3%	∼(AIN-76)*	∼(AIN-76)*	0.1%	Athauda *et al.* (2004)
LP 9%	Crude protein 9%	5%	8%	Cerelose 68.17%	Soy oil 4.3%	R/M 3 premix 5.45%	R/M 3 premix 5.45%	0.08%	Holness & Sugden (1999)
C 22%	Crude protein 22%	5%	8%	Cerelose 55.15%	Soy oil 4.3%	R/M 3 premix 5.05%	R/M 3 premix 5.45%	0.2%	Holness & Sugden (1999)

All percentage values are given as w/w. *Total vitamin/mineral mix is 3.5%. Note that due to varying moisture and nitrogen free extract (polysaccharide) content (not given in original papers), rows may not sum to 100%. Abbreviations: SH, Southampton low protein diet; SHC, Southampton control diet; HF, Hope Farm low protein diet; HFC, Hope Farm control diet. Other diets are referred to by the percentage of protein where C denotes control and LP denotes the low protein diet. Note that although vitamin and mineral pre-mixes vary subtly, all are similar to the AIN-76 formulation.

Table 3. Commonly used high fat feeding protocols

Diet	Protein	Starch	Simple sugars	Fibre	Lipid	Vitamins	Minerals	Methionine	Reference
HVF 40%	Casein 15%	15%	Sucrose 15%	5%	Veg oil 40%	(AIN-76) 1%	(AIN-76) 3.5%	NA	Guo & Jen (1995)
C 4%	Crude 23.4%	15%	Sucrose 15%	5%	Corn oil 5.3%	(AIN-76) 1%	(AIN-76) 3.5%	NA	Guo & Jen (1995)
HAF 24%	Crude 19.5%	35.8%	Sucrose 5.4%	4.5%	Corn oil 4.3%, Lard 20%	R/M 3 Premix 2.6%	R/M 3 Premix 2.6%	0.2%	Khan *et al.* (2003)
C 4%	Crude 21.6%	48.8%	Sucrose 5.4%	4.5%	Corn oil 5.3%	R/M 3 Premix 1.6%	R/M 3 Premix 1.6%	0.2%	Khan *et al.* (2003)
HSF 17.4%	Crude 15.4%	—	43.3%	5%	Coconut oil 17.4%	(AIN-93G) 3%	(AIN-93) 1.1%	NA	Siemelink *et al.* (2002)
HFF 18.1%	Crude 15.5%	—	44.5%	5%	Menhaden oil 18.1%	(AIN-93G) 3%	(AIN-93) 1.1%	NA	Siemelink *et al.* (2002)
HUF 18.4%	Crude 15.8%	—	42.5%	5%	Soy oil 18.4%	(AIN-93G) 3%	(AIN-93) 1.1%	NA	Siemelink *et al.* (2002)
HAF22%	19% (88% casein)	34%	—	17.9%	Corn oil 1.9%, Lard 20.1%	R/M 3 Premix 5.01%	R/M 3 Premix 5.01%	0.4	Holness & Sugden (1999)
C 4%	22% (88% casein)	8%	Cerelose 55.1%	5%	Soy oil 4.3%	R/M 3 Premix 5.05%	R/M 3 Premix 5.05%	0.2	Holness & Sugden (1999)

All percentage values are given as w/w. *Total vitamin/mineral mix is 3.5%. Note that due to varying moisture and nitrogen free extract (polysaccharide) content, rows will not sum to 100%. Abbreviations: HVF, vegetable oil based high fat diet; HAF, high animal fat diet; HFS, high saturated fat diet; HFF, high fish oil diet; HUF, high unsaturated fat diet; NA, data not available from reference. Note that although vitamin and mineral pre-mixes vary subtly, all are similar to the AIN-76 formulation.

been undertaken in the rat. Almost invariably, and unless stated in the text, offspring from these protocols have been weaned onto a normal laboratory chow diet and maintained on this diet until study in adulthood.

The majority of studies of nutrient restriction have employed a 50% reduction in dietary protein. Although individual laboratories generally adhere to one nutrient regime, a variety of diets and protocols have been reported. Most often it is the proportions of macronutrients in these experimental diets that differ, particularly the source of carbohydrate (for example some use starch as a major carbohydrate source, others use glucose) and the lipids supplied (some diets are essential fatty acid deficient) (Table 2). This results in widely varying protein : lipid : carbohydrate ratios. Additionally, the timing and duration of maternal dietary manipulation varies between groups (Tables 4–7). Most notably, although many restriction studies aimed to mimic low birthweight and/or catch up growth, few have achieved it. In the protein restriction models, birthweight may be lowered (Galler & Tonkiss, 1991; Langley & Jackson, 1994; Holemans *et al.* 1999; Vickers *et al.* 2001*a*,*b*; Woods *et al.* 2001), although there are reports of increased birthweight (Langley-Evans *et al.* 1996*b*) or no effect of the maternal diet on offspring birthweight (Langley & Jackson, 1994). Perhaps unsurprisingly, the adult phenotypes arising from the various restriction protocols also vary in some important respects.

Rodent studies of dietary restriction have also been undertaken. Models range from mild (30% reduction in caloric intake: Ozaki *et al.* 2001), through moderate (50% reduction in caloric intake: Holemans *et al.* 1999) to severe (70% reduction in caloric intake: Woodall *et al.* 1996; Vickers *et al.* 2001*b*). Offspring of dams exposed to caloric restriction during pregnancy are generally born with low birthweight (Woodall *et al.* 1996; Holemans *et al.* 1999; Ozaki *et al.* 2001; Franco *et al.* 2002*b*, 2003). There is no evidence of adult obesity, but catch up growth does occur in some models (Ozaki *et al.* 2001; Franco *et al.* 2002*b*).

In ovine models the predominant dietary restriction protocol used is global caloric reduction. The majority of studies to date have only observed consequences for fetal physiology since the ability to catheterize fetal sheep uniquely permits studies on the effects of a suboptimal intrauterine environment directly on the fetus (Hawkins *et al.* 1999, 2000; Ozaki *et al.* 2000; Edwards & McMillen, 2001). These are not addressed in detail as this review focuses on the adult phenotype. Relatively few studies address the possibility of developmental programming of features of the metabolic syndrome in postnatal animals. Fifty per cent global nutrient restriction during the first half of pregnancy with subsequent realimentation does not reduce neonatal lamb weight but results in altered development of organs at birth such as the liver and heart, which may be key to the later development of the metabolic syndrome (Vonnahme *et al.* 2003). Offspring of ewes fed 50% of the food intake of control ewes from 1 to 30 days (term approximately 147 days) with an adequate diet thereafter showed no change in birth weight or postnatal growth to 1 year of age (Gardner *et al.* 2004).

The effect of maternal iron deficiency on rat offspring has also been studied, though to a lesser extent than protein or caloric deprivation, and there is evidence for developmental programming of several aspects of the metabolic syndrome in offspring reared on a normal diet following maternal iron deprivation. This model of iron deficiency bears relevance to human dietary conditions throughout the world, as irrespective of social status or food availability, maternal anaemia is manifest; for example 13% of German women develop clinical anaemia (haemoglobin < 11 g dl^{-1}) during pregnancy (Bergmann *et al.* 2002) and a study of pregnant women attending antenatal clinics (34 week visit) in the United Kingdom has reported iron deficiency in approximately 30% of subjects (Fosset *et al.* 2004). Three protocols to induce iron deficiency in rats have been utilized; Gambling *et al.* (2003) fed dams either control (50 mg kg^{-1} $FeSO_4$) or iron deficient (7.5 mg kg^{-1} $FeSO_4$) diets 4 weeks prior to mating and through gestation, Lisle *et al.* (2003) provided control (153 mg kg^{-1} iron subcarbonate) or iron deficient (3 mg kg^{-1} iron subcarbonate) diets for 1 week before mating and throughout gestation, whilst Lewis *et al.* (2001) provided control (150 mg kg^{-1} iron subcarbonate) or iron deficient (3 mg kg^{-1} iron subcarbonate) diets for 1 week prior to mating and throughout gestation. Birthweight was reduced in male, but not female, offspring of iron-deprived Rowett Hooded Lister rats (Gambling *et al.* 2003) but catch-up growth occurred by 6 weeks of age. However, male and female offspring of iron-deprived Wistar dams were growth restricted at 2–3 days of age (Lewis *et al.* 2002; Lisle *et al.* 2003) but did not display catch-up growth at 14 weeks (Lewis *et al.* 2002) or 18 months (Lisle *et al.* 2003) of age.

Caloric or protein restriction protocols may mimic the challenge faced in developing nations, or in the underprivileged members of Western societies but have little in common with the dietary intake of the majority of Western societies. Several groups have therefore investigated the effects of a diet high in fat or cholesterol, which may be more relevant to the average Western diet and may give insight into potential mechanisms for the aetiology of cardiovascular disease (Napoli *et al.* 2000; Ghosh *et al.* 2001; Palinski *et al.* 2001; Khan *et al.* 2003, 2004). Table 3 describes the composition of commonly used maternal fat-rich diets. Animals exposed to high saturated fat during gestation and suckling may have birth weights that are lower than controls (Langley-Evans, 1996), or the same as controls (Siemelink *et al.* 2002). Adult weight is reported to be the same as controls at 12 and (Siemelink *et al.* 2002), 26 weeks of age (Khan *et al.* 2003).

Table 4. Offspring insulin and/or glucose homeostasis in dietary models of developmental programming in the rat

Strain	Protocol	Intervention period (day of gestation)	Age at measure (weeks)	Sex studied	Parameter measured and outcome	Abnormal result	Reference
SHR	50% of ad lib	1–22	13–16 w	M + F	Plasma glucose ↑	Yes M	Franco *et al.* (2002*b*)
Wistar	50% of ad lib	15–21	3 w	C	Islet insulin content ↓	Yes	Garofano *et al.* (1997)
Wistar	50% of ad lib	11–22	3, 11 w	F	Plasma insulin↓ glucose↑	Yes	Holemans *et al.* (1999)
Wistar	70% of ad lib	0–18	28 w	M + F	Plasma glucose↑	Yes M	Ozaki *et al.* (2001)
Wistar	30% of ad lib	0–22	17 w	F	Plasma insulin ↑	Yes	Vickers *et al.* (2001)
Wistar	IR	−7–21	12 w	M + F	GTT insulin sensitivity↑	No M + F	Lewis *et al.* (2001)
Wistar	IR	−7–21	64 w	M + F	GTT insulin sensitivity ↔	No M + F	Lewis *et al.* (2002)
SD	HF 8% protein	0–43	10 w	C	GTT insulin sensitivity ↓	Yes	Dahri *et al.* (1991)
Wistar	HF 8% protein	0–43	6 w	C	GTT insulin sensitivity ↓	Yes	Shepherd *et al.* (1997)
Wistar	HF 8% protein	0–43	44 w	C	Glucokinase expression↓	Yes	Shepherd *et al.* (1997)
Wistar	SH 6% protein	−14–22	9 w	F	GTT insulin sensitivity ↓	Yes	Langley *et al.* (1994)
Wistar	HF 8% protein	0–43	68 w	M	GTT insulin sensitivity ↓	Yes	Petry *et al.* (2001)
SD	40% saturated fat	−7–22	3 w	C	Plasma glucose: insulin ↑	Yes	Guo & Jen (1995)
SD	24% saturated fat	−10–22	25 w	M + F	Plasma glucose ↑	Yes F	Khan *et al.* (2003)
SD	24% saturated fat	−10–43	25 w	F	Euglycaemic-hyperinsulinaemic clamp, ISI ↓	Yes	Taylor *et al.* (2004)
SD	18% saturated fat	−14–43	12 w	M	GTT insulin sensitivity ↓, Islet cells ↓	Yes	Siemelink *et al.* (2002)

Timing of protocol is relative to day of gestation (birth is at 21–22 days in a rat); −10 indicates 10 days prior to mating and 43 days is equivalent to offspring post natal day 21 (weaning). Abbreviations: SHR, spontaneously hypertensive rat; SD, Sprague-Dawley rat; IR, iron restricted diet (iron content 3 mg (kg diet)$^{-1}$); GTT, glucose tolerance test; HF, Hope Farm low protein diet; SH, Southampton low protein diet; w, postnatal week; ↑, increased; ↓, decreased; ↔, no change; M, male only; F, female only; C, combined male and female; M + F, both male and female considered individually.

The duration and timing of the dietary intervention varies between groups (see Tables 4–7 for timing details). Many studies manipulate diet only until the end of pregnancy but despite the improved diet provided during suckling, animals do not invariably demonstrate 'catch-up' growth. This may reflect variations in the severity of particular protocols. In humans catch-up growth, or more specifically departure from the normal range of growth trajectory, appears to be an important predictor of later adult disease. Data from animal studies suggest therefore that the relationship between altered growth trajectory and adult disease does invariably exist as animals may be born with normal birth weight and demonstrate the same phenotype (e.g. raised blood pressure) as animals born of low birth weight with 'catch-up' growth. This does not necessarily relate to lifespan as Ozanne & Hales (2004) have recently shown animals showing 'catch-up' growth to have a reduced longevity compared with animals that do not show 'catch-up' growth.

Remarkably, there is evidence for the programming of certain features of the metabolic syndrome from both restriction (calorie, protein and iron) and fat-feeding studies, possibly suggesting a commonality of mechanism and highlighting that balanced maternal nutrition is vital to subsequent offspring health. It should, however, be noted that the majority of studies have investigated offspring weaned onto and maintained on a normal balanced diet of laboratory chow. Few studies have manipulated the postnatal diet and the 'thrifty phenotype' and predictive adaptive response hypotheses remain to be adequately tested.

Developmental programming of metabolic syndrome by maternal dietary challenge

The following sections address evidence for the key criteria of the metabolic syndrome, insulin resistance, hypertension and dyslipidaemia and also of endothelial function in the different nutritional and placental insufficiency models of developmental programming.

Insulin resistance. Table 4 shows evidence for perturbation of insulin and glucose homeostasis in dietary models of developmental programming. During periods of poor maternal nutrition, the fetus diverts nutrients to critical organs, particularly the brain, at the expense of visceral organs, especially the pancreas and liver. Accordingly, structural changes and enzymatic alterations in these two organs have been extensively studied in the low protein model. Offspring of animals exposed to different protein and calorie restriction protocols and at different periods of gestation have slightly differing adult phenotypes; however, all appear

to programme abnormalities of the fetal pancreas. It is presumed that these changes in islet morphology contribute to the increased susceptibility to adulthood insulin resistance.

Protein restriction. A large body of work supports the hypothesis that structure and function of the fetal pancreas are altered following maternal protein restriction induced by the 'Hope Farm' diet (see Table 2; Snoeck *et al.* 1990; Sener *et al.* 1996; Petrik *et al.* 1999). Recently, Sparre *et al.* (2003) showed altered protein expression in islet cells from term fetuses exposed *in utero* to the 'Hope Farm' diet. At gestation day 21.5, gene products found (by gene array comparison) to be down regulated in pancreatic islet cells included those involved in mitochondrial respiration, antioxidant defences and glucagon metabolism (Sparre *et al.* 2003). In adult rat kidney and muscle (5–20 weeks of age), reduced mitochondrial copy number as well as expression of genes involved in mitochondrial respiration have also been observed (Park *et al.* 2003). Reduced mitochondrial copy number is found in peripheral blood leucocytes of humans, before the development of clinical diabetes mellitus (Lee *et al.* 1998). Of relevance to developmental programming, lowered mitochondrial copy number within peripheral leucocytes appears to correlate with both low birthweight and decreased adult glucose tolerance (oral glucose tolerance test (GTT)) (Lee, 1999). Furthermore, non-diabetic offspring from diabetic parents show reductions in mitochondrial copy number (Song *et al.* 2001).

Oral glucose tolerance at 70 days of age was impaired in offspring of Sprague-Dawley rats fed the 'Hope Farm' diet (Dahri *et al.* 1991). Glucose intolerance was associated with a diminished insulin secretory response to an oral glucose tolerance load and the abnormal insulin response was retained into adulthood (Dahri *et al.* 1991). There is also evidence for accelerated age-related dysfunction, or islet cell insulin depletion, as younger offspring of Sprague-Dawley dams fed an 8% protein diet demonstrated better glucose regulation than controls (oral tolerance test), but underwent a greater age-dependent loss of glucose tolerance than controls to become glucose intolerant at 1 year (Petry *et al.* 1997; Shepherd *et al.* 1997). In male offspring this glucose intolerance was ascribed to insulin resistance whereas, in females, glucose intolerance was reported to be due to insulin deficiency (Shepherd *et al.* 1997), suggesting possible sex-specific mechanisms. Langley *et al.* (1994) utilizing the glucose tolerance test also demonstrated impaired glucose tolerance in adult offspring of Wistar rats fed the 'Southampton' low protein diet (9% protein, control is 18%).

In a study in which offspring of protein restricted rats have been reared onto a low protein diet and maintained on that diet to adulthood, Dahri *et al.* (1991) have shown that lifelong protein restriction in Wistar rats produces a similar phenotype (impaired oral GTT) to that of offspring exposed to the protein deficient diet during gestation only. Although these data support the notion that protein restriction in development permanently programmes adult dysfunction, the predictive adaptive response and thrifty phenotype hypotheses would predict that lifelong protein restricted animals would demonstrate normal glucose tolerance, as the animals were receiving the diet they adapted to during development. In contrast, Holness & Sugden (1999) have studied rats subject to lifelong protein restriction and these animals were found to have enhanced insulin sensitivity by euglycaemic–hyperinsulinaemic clamp, but when transferred to a fat rich diet, the animals developed insulin resistance over and above that of control animals fed the fat diet (Holness & Sugden, 1999). This would provide support to the predictive adaptive response hypothesis, in which these rats appeared to be pre-conditioned for survival on the same diet as their dam, but in doing so were made vulnerable to the adverse influences of a high calorific diet. There are therefore insufficient and conflicting data upon which to critically examine whether lifelong protein restriction in rats results in a phenotype that is consistent with the predictive adaptive response hypothesis.

Alterations to the function (either due to reduced expression or activity) of hepatic enzymes which play a key role in glucose homeostasis have been reported in the 'Hope Farm' low protein model (Desai *et al.* 1997). Thus it has been proposed that programming of adult offspring glucose tolerance may be a result of altered expression of hepatic insulin-sensitive enzymes. Indeed, a twofold reduction in glucokinase and a twofold increase in phosphoenolpyruvate carboxykinase has been observed in offspring of protein restricted dams at 11 months of age. This altered carbohydrate metabolism was attributed to reduced *in utero* protein exposure (Desai *et al.* 1997), but because glucose was used to replace protein in this diet (Table 2), the programming stimulus could equally be a carbohydrate overload as protein restriction.

Caloric restriction. Moderate maternal food restriction (30% reduction in *ad libitum* intake) in guinea-pigs resulted in glucose intolerance (intravenous GTT) in adult male offspring, and raised plasma insulin was observed in adult male offspring of guinea-pig sows subjected to mild (15% reduction of *ad libitum* intake) restriction (Kind *et al.* 2003). Severe (50%) food restriction, imposed from day 15 of pregnancy in Wistar rats induced intrauterine growth restriction in the offspring. From birth, β-cell mass and insulin content were significantly decreased in the growth-retarded pups compared with controls. Although catch-up growth occurred by weaning, β cell mass and insulin content remained low, despite provision of adequate nutrition, and this dysfunction worsened

with age (Garofano *et al.* 1997, 1998*a*,*b*, 1999). Garofano *et al.* (1998*a*,*b*) suggest that dietary restriction during the weaning period is most relevant to the development of altered pancreatic insulin status in adulthood; however, it is clear that earlier severe restriction (70%) also results in altered fetal plasma insulin (Woodall *et al.* 1996), and protein restriction results in altered fetal pancreatic structure and function (Snoeck *et al.* 1990; Sener *et al.* 1996; Petrik *et al.* 1999) so the fetal pancreas is clearly affected.

Iron restriction. Offspring of iron restricted Wistar dams showed improved glucose tolerance compared with control offspring (intraperitoneal GTT) at 3 months of age (Lewis *et al.* 2001) but by 18 months of age there was no difference in glucose tolerance between control and iron restricted offspring (Lewis *et al.* 2002), which may reflect an accelerated decline in glucose tolerance. However, older animals are yet to be studied, so this cannot be verified. Moreover, Gambling *et al.* (2003) reported no difference in oral GTT between 10-week-old offspring of iron restricted and control Rowett Hooded Lister dams.

Fat feeding. Offspring hyperglycaemia and hyperinsulinaemia (Gerber *et al.* 1999; Khan *et al.* 2003, 2004) are also observed after exposure to a maternal diet rich in animal lard. This profile is associated with a reduced insulin secretory capacity of pancreatic (β) islet cells at 6 months of age, and whole body insulin resistance assessed by the euglycaemic–hyperinsulinaemic clamp (Taylor *et al.* 2004). In another study of 12-week-old offspring of dams fed a fat-rich diet in pregnancy, an increased insulin secretory response to an oral glucose load (insulin resistance) was observed in offspring of dams fed a lard-rich diet. However, offspring glucose tolerance was normal when the maternal fat intake was derived from polyunsaturated fat rich fish oil (Siemelink *et al.* 2002), suggesting that saturated fat intake *per se* may be the programming stimulus. These studies suggest that early β cell over-stimulation may result in islet cell insulin depletion in these models that manifests as frank insulin deficiency later in life, as occurs in the development of type 2 diabetes in man. In addition, we (Taylor *et al.* 2004) have shown reduced mitochondrial copy number in kidney from 6-month-old Sprague-Dawley offspring of fat-fed dams. This, along with other findings of mitochondrial dysfunction in dietary challenge and the association with low birthweight and type 2 diabetes mellitus (Lee *et al.* 1998; Lee, 1999; Song *et al.* 2001; Park *et al.* 2003) may represent a mechanism for the development of particular facets of the metabolic syndrome. Litter size, and presumably increased milk availability during suckling, also has an effect on glucose homeostasis, whereby adult rats from small litters (3–4 pups) show increased plasma insulin concentrations compared with adult rats from large litters (14–24 pups) that might have received a less optimal milk supply during suckling due to competition from littermates (Hahn, 1984).

Although many studies report 'insulin resistance' or reduced 'insulin sensitivity' in offspring of dietary challenged animals, there is considerable variability amongst the methods employed. The oral glucose tolerance test is most often used, and most likely represents the hepatic/pancreatic response to the glucose load. Few studies have as yet utilized the euglycaemic–hyperinsulinaemic clamp, which is the preferred method for determining whole body insulin sensitivity, and reflects peripheral (skeletal muscle) insulin sensitivity. One of the groups that have adopted this approach, Ozanne and colleagues, have demonstrated evidence for superior insulin sensitivity (enhanced glucose uptake from isolated skeletal muscle) in 3-month-old offspring of protein deprived rat dams (Ozanne *et al.* 1996) but insulin resistance (by euglycaemic–hyperinsulinaemic clamp) in 15-month-old offspring of protein deprived rats (Ozanne *et al.* 2003). Importantly, intracellular signalling pathways involved in glucose uptake have been shown to be modified in insulin-sensitive tissue from offspring of protein restricted dams; thus Ozanne *et al.* (2003) have shown reduced skeletal muscle expression of the protein kinase C zeta isoform (PKCζ), one of the family of protein kinases recently implicated in intracellular insulin signalling pathways.

In summary, offspring insulin resistance, a hallmark of human metabolic syndrome, is consistently programmed by maternal dietary imbalance. Changes to pancreatic structure and function are observed in fetal life, and these alterations persist to adulthood. Altered mitochondrial copy number has been observed in fat-rich and protein restriction models and may provide a mechanism for the observed cellular dysfunction. In adult offspring, insulin or glucose sensing in pancreatic islet β cells, liver and skeletal muscle are patently defective in the animal models and data from longitudinal studies suggest a resemblance to the progression of type 2 diabetes in man. Further assessment of intracellular pathways, and greater application of the euglycaemic–hyperinsulinaemic clamp in offspring of dietary challenged dams will further verify what appears to be robust developmental programming models of abnormal insulin homeostasis.

Blood pressure

Hypertension in man is characterized by predefined values above the 'norm', which are associated with adverse outcome, whereas in the rat the term 'hypertension' is often used to describe a statistically significant increase in blood pressure from control. As a result of the variety in blood pressure measurement techniques, strain to strain and sex differences, it is difficult to define set values for 'hypertension' in the rat as basal blood pressure

Table 5. Offspring blood pressure in dietary models of developmental programming in the rat

Strain	Protocol	Intervention period	Age at measure (day of gestation)	Sex studied	Measurement technique	Higher than control?	Reference
Wistar	50% of ad lib	1–22	16 w	M + F	Tail cuff	Yes M	Franco *et al.* (2003)
SHR	50% of ad lib	1–22	13–16 w	M + F	Tail cuff	Yes M + F	Franco *et al.* (2002*b*)
Wistar	50% of ad lib	11–22	14–17 w	F	Femoral artery catheter	N	Holemans *et al.* (1999)
Wistar	70% of ad lib	0–18	28 w	M + F	Femoral artery catheter	Yes M + F	Ozaki *et al.* (2001)
Wistar	30% of ad lib	0–22	50 w	M + F	Tail cuff	Yes M + F	Woodall *et al.* (1996)
Wistar	30% of ad lib	0–22	25 w	F	Tail cuff	Yes	Vickers *et al.* (2001)
Wistar	IR	−7–22	12 w, 64 w	M + F	Tail cuff	Yes M + F	Lewis *et al.* (2001); Lewis *et al.* (2002)
RHL	IR	−28–22	10 w	M + F	Tail cuff	Yes M + F	Gambling *et al.* (2003)
Wistar	SH6%	−14–22	21 w	F	Tail cuff	Yes	Langley & Jackson (1994)
Wistar	SH9%	−14–22	21 w	F	Tail cuff	Yes	Langley & Jackson (1994)
Wistar	SH12%	−14–22	21 w	F	Tail cuff	No	Langley & Jackson (1994)
Wistar	SH9%	0–22	7 w	M + F	Tail cuff	Yes M + F	Langley-Evans *et al.* (1996*b*)
Wistar	SH9%	0–22	18 w	M	Tail cuff	Yes	Brawley *et al.* (2003)
SD	LP8.5%	−0–22	20 w	M	Femoral artery catheter	Yes	Woods *et al.* (2001)
SD.	LP6%	−35–22	13 w	M	Radiotelemetry	No	Tonkiss *et al.* (1998)
SD	HF8%	0–93	52 w	F	Tail cuff	Yes	Petry *et al.* (1997)
Wistar	LP6%, 9%	0–22	10–13 w	NA	Tail cuff	Yes	Lamireau *et al.* (2002)
SD	24% saturated fat	−10–43	25 w	M + F	Radiotelemetry	Yes F	Khan *et al.* (2003; 2004)
SD	10% fat *n*–3 PUFA deplete	0–63	36 w	M + F	Femoral artery catheter	Yes M + F	Weisinger *et al.* (2001)
Wistar	9% saturated fat	−14–22	7 w	F	Tail cuff	Yes	Langley-Evans (1996)

Timing of protocol is relative to day of gestation (delivery is at 21–22 days in a rat); −10 indicates 10 days prior to mating and 43 days is equivalent to offspring postnatal day 21 (weaning). Abbreviations: SHR, spontaneously hypertensive rat; RHL, Rowett Hooded Lister rat; SD, Sprague-Dawley rat; IR, iron restricted diet (iron content 3 mg kg^{-1}); HF, Hope Farm low protein diet; SH, Southampton low protein diet; LP (%), low protein diet (% protein content); w, postnatal week; M, male only; F, female only; C, combined male and female; M + F, both male and female considered individually.

varies substantially. For example, control systolic blood pressure values measured by tail cuff plethysmography in Wistar rats have been reported to range between 75 and 150 mmHg in the same study (Petry *et al.* 1997).

Table 5 presents the evidence for raised blood pressure, in the different animal models, together with measurement techniques. Amongst the different protocols used to assess the responses to maternal protein restriction, the reports of offspring blood pressure, as for birthweight, are inconsistent.

Protein restriction. The 'Hope Farm' diet does not appear to lead to raised blood pressure in chow fed adult offspring except in one study in which offspring were fed the restricted diet until 93 days of age, then standard chow until 1 year of age, when the animals demonstrated increased systolic blood pressure (Petry *et al.* 1997). Adult Wistar offspring of dams fed the 'Southampton' diet (9% protein, control is 18%) generally had raised systolic blood pressure, as measured by the tail cuff method, with differences from control as high as 35 mmHg (Sherman & Langley-Evans, 2000). Elevation of blood pressure in young offspring has been found after exposure to a 12% protein maternal diet; however, by 26 weeks of age this difference had disappeared (Langley *et al.* 1994) (Table 3). Woods *et al.* (2001), using a diet with lower carbohydrate content than the 'Hope Farm' diet and several compositional differences from the 'Southampton' diet (Table 2), showed an increase in mean arterial pressure of 10 mmHg (by arterial cannulation in conscious Sprague-Dawley rats) in 20-week-old offspring exposed to a 9% low protein diet (Table 5) *in utero* and during suckling. Offspring of Sprague-Dawley rats fed a 66% reduced protein diet from day 12 of gestation to term, then reared on a normal protein diet, demonstrated increased (40 mmHg) systolic blood pressure compared with controls by tail cuff plethysmography measurement from 8 weeks of age onwards (Vehaskari *et al.* 2001). Aihie Sayer *et al.* (2001), Vehaskari *et al.* (2001) and Ozanne *et al.* (2004) investigated offspring of protein restricted pregnant rats and mice. Here there is some variability in results as Vehaskari *et al.* (2001) and Aihie Sayer *et al.* (2001) demonstrated reduced lifespan in rats exposed to low protein diets during gestation, whereas Ozanne & Hales (2004) have shown such a reduction in lifespan as a result of maternal malnutrition during the suckling period.

Whilst the composition of the protein restriction diets, which primarily differ in simple sugars, lipids and methionine (see below), is the most obvious reason for the wide range of increments in blood pressure, some variability could result from differences between protocols, primarily the duration of feeding of the low protein diet. In earlier studies the maternal diet was provided 7 or 14 days prior to pregnancy and throughout gestation (Langley *et al.* 1994; Langley-Evans & Jackson, 1995) whereas in latter studies the diet was fed throughout pregnancy only (Langley-Evans, 1996). Adult offspring of dams habituated to the low protein diet 14 days prior to pregnancy showed a higher systolic blood pressure compared with those fed the diet during pregnancy only (Langley-Evans *et al.* 1996*b*) indicating a pre-pregnancy programming stimulus, or merely reflecting a dose/duration-dependent effect of the low protein diet. The differences in blood pressure may also partly be attributable to an age dependency of the response as blood pressure was assessed at different ages from weaning until 21 weeks of age, and in one study at 1 year. As shown in Tables 4–7, attention paid to sex differences has been variable. Some studies report combined male and female data whereas others focus on one sex alone. Given that there are clear sex differences in baseline blood pressure between the male and female rats and increasing evidence of sex differences in developmentally programmed animals, future studies should always address sex differences.

Caloric restriction. Female offspring of Wistar rats fed a severely reduced (30% of *ad libitum* intake) diet in pregnancy then suckled and reared on a normal diet are reported to have increased systolic blood pressure by tail cuff measurement at 15 (Vickers *et al.* 2000) and 30 weeks of age (Woodall *et al.* 1996), and the blood pressure remains elevated with age. Others have found that Wistar dams subjected to 50% of *ad libitum* intake also produce male offspring with adulthood elevation of systolic blood pressure by tail cuff (Franco *et al.* 2002*a*). Furthermore, pregnant Wistar rats fed 70% of an *ad libitum* diet through most of gestation (0–18 days) produced male offspring in which mean arterial blood pressure, as assessed by arterial cannulation, in conscious animals was raised by 60 days and increased with age compared with age matched controls (Ozaki *et al.* 2001). Female offspring of caloric restricted dams developed significantly increased blood pressure by 100 days of age in the same study, indicating sex differences within this model (Ozaki *et al.* 2001). Three-year-old male offspring of sheep subject to 50% caloric restriction in the first and second trimester of pregnancy demonstrated altered baroreflex responses compared with controls (Gopalakrishnan *et al.* 2004). Moreover, these offspring of calorie-restricted ewes had increased pre-prandial systolic and diastolic blood pressure as well as increased heart rate compared with controls but post-prandially these differences were not different from controls. The authors suggest that the increased blood pressure and heart rate observed pre-prandially might be due to increased circulating leptin concentrations leading to increased sympathetic nervous system activation (Gopalakrishnan *et al.* 2004). Maternal undernutrition (50% reduction in calories) in the peri-implantation period (first 30 days of gestation) results in normal resting blood pressure but increased pulse pressure and a leftward shift in the baroreflex in 1-year-old sheep (Gardner *et al.* 2004). In one of the few studies in guinea-pigs, Kind *et al.* (2002) have shown that male offspring of 30% calorie restricted sows to have statistically significant increases (8 mmHg) in systolic blood pressure, measured by indwelling carotid artery cannulation at 15 weeks of age.

Iron restriction. Offspring of iron deficient dams, reared on laboratory chow from weaning onwards, are also reported to demonstrate increased blood pressure. Tail cuff plethysmography was used in all studies. Lewis *et al.* (2001) reported significantly increased systolic blood pressure at 3 months in male offspring of iron restricted Wistar dams (Lewis *et al.* 2001). By 16 months of age, both males and females showed increased systolic blood pressure compared with controls (Lewis *et al.* 2002). In a repeat study, using very similar diets, Lisle *et al.* (2003) show increased systolic blood pressure in both male and female offspring of iron restricted dams at both 3 and 18 months of age that was accompanied by a reduction in nephron number (Lisle *et al.* 2003). At 6 weeks of age, male offspring of iron restricted Rowett Hooded Lister dams had higher systolic blood pressure than controls, and by 10 weeks of age, both male and female offspring of iron restricted dams showed increased systolic blood pressure compared with offspring of control dams (Gambling *et al.* 2003). These findings are similar to those from protein restriction protocols, and interestingly the diet base used is very similar to that of the 'Hope Farm' control diet (Table 2), so although the 'Hope Farm' low protein diet does not produce blood pressure increases in offspring, iron deficiency superimposed upon the 'Hope Farm' control diet does result in alterations to blood pressure.

Fat feeding. Using radiotelemetric recording in conscious unrestrained animals, Khan *et al.* (2003) reported raised (approximately 10 mmHg) systolic and diastolic blood pressure in 6- and 12-month-old female offspring of dams fed a diet rich in animal lard. This increase was not observed in males. Interestingly, heart rate in males (but not females) was lower, suggesting that a loss of baroreceptor sensitivity in females, or a difference in baroreceptor ability to compensate in males, may underlie the raised blood pressure (Khan *et al.* 2003, 2004).

Using a saturated fat supplemented maternal diet (10% coconut oil), Langley-Evans (1996*a*) showed an effect of maternal saturated fat feeding on adult offspring systolic blood pressure (37 mmHg by tail cuff), but maternal polyunsaturated fatty acid intake (10% corn oil) did not result in elevation of blood pressure – indicating that certain fatty acids may be more deleterious than others. Indeed, omega-3 polyunsaturated fatty acid deprivation (10% safflower oil) from conception to 9 weeks of age produced a maintained (measured at 33 weeks of age) elevation of mean arterial blood pressure in conscious unrestrained Sprague-Dawley rats of up to 17 mmHg (Weisinger *et al.* 2001; Armitage *et al.* 2003). It is feasible that increased blood pressure observed in the adult offspring subjected to some of the maternal low protein restriction protocols could, at least partially, be the result of omega-3 fatty acid deficiency as the corn oil used as the lipid source is omega-3 polyunsaturated fatty acid (PUFA) deficient (Tables 2 and 3).

Methodological considerations regarding blood pressure measurement

Importantly, as most studies of developmental programming of raised blood pressure have used tail cuff plethysmography, which involves mild restraint, there is an added possibility that the 'basal' blood pressure recorded may sometimes not be a true baseline recording, but be variably elevated in response to restraint. Animals are generally 'trained' by restraint or handling to minimize stress, which may explain the equivalence in baseline values achieved in some studies using the tail cuff system to those achieved by telemetry or direct cannulation in conscious animals. The tail cuff technique will, however, always be prone to a variable influence of restraint (Irvine *et al.* 1997; Jamieson *et al.* 1997). Accuracy of the method is also prone to influences of measurement artefacts, introduced by temperature-induced increases in blood flow, as animals require heating to achieve adequate tail vessel dilatation. Furthermore, different measurement systems (commercial or bespoke) require that the animals be maintained at temperatures that vary from quite mild increases (animal heating to 27°C) to direct warming of the tail with an infra-red heat lamp. Raised systolic blood pressure in offspring in early life has tended to decrease with age in certain protein restriction studies (Langley & Jackson, 1994), which might indicate transient 'programming', or could reflect a decline in a programmed sympathetic stress response with age, since sympathetic activity declines with age in normal rats (Lakatta, 1980; Weisfeldt, 1998; Stratton *et al.* 2003). One study has directly implicated developmental programming of an exaggerated stress response. Tonkiss *et al.* (1998) evaluated the effect of a stress induced by exposure to ammonia odour in male offspring of dams fed a 9% casein diet with the technique of radio-telemetry (Tonkiss *et al.* 1998). Basal blood pressure was similar in controls and offspring of dams fed the low protein diet. However, upon the stressor test, offspring of dams fed the low protein diet displayed significantly greater increases in systolic and diastolic blood pressure than controls. This is strongly suggestive of the maternal low protein diet leading to alteration in the offspring sympathetic axis (Tonkiss *et al.* 1998). Altered sympathetic nervous system function (increased pulse pressure and a leftward shift in the baroreflex) has been also reported in chronically instrumented 1-year-old sheep offspring of dams fed a 50% reduction in calories for the first 30 days of gestation (Gardner *et al.* 2004).

In summary, raised blood pressure, one of the key criteria for metabolic syndrome, occurs in the majority of rat developmental models of maternal dietary intervention. The notable exception is the 'Hope Farm' diet, used by many investigators, which does not routinely induce elevation of blood pressure in offspring raised on a normal diet. However few studies have challenged offspring of nutritionally challenged animals with an adverse, high calorie or high protein diet in adulthood, which according to the predictive adaptive response hypothesis would exacerbate developmental programming of cardiovascular dysfunction in offspring of caloric or protein restricted dams. Further studies to test the hypothesis are required.

Central adiposity

Central adiposity is considered to be pivotal to the increase in the incidence of the metabolic syndrome in human populations. Table 6 shows the evidence for obesity in developmental programming models. Unlike the epidemiological studies in low birthweight individuals, investigations in the rat employing maternal caloric, protein or iron restriction do not, on the whole, offer evidence for adult obesity in adult offspring fed a normal diet after weaning. The 'gold-standard' measurement technique is DEXA MRI scanning; however, fat pad weights or whole body composition (by combustion and ash analysis) also yield useful information about body fat deposition.

Protein/caloric/iron restriction. In rat offspring from protein, calorie and iron deprivation protocols there is no evidence for an increase in bodyweight and, in fact, limited data point to decreased adult fat depots in protein restriction studies in rats reared on a normal diet (Shepherd *et al.* 1997), although Kind *et al.* (2003) reported increased retroperitoneal fat pad mass in adult male offspring of guinea-pigs fed a reduced caloric intake (70% of *ad libitum* intake) and Vickers *et al.* (2000, 2001*a*) reported increased retroperitoneal and gonadal fat pad size (relative to percentage body weight) in male and female

Table 6. Other facets of metabolic syndrome in offspring from dietary models of developmental programming in the rat

Strain	Protocol	Intervention period (day of gestation)	Age at measure	Sex studied	Parameter measured	Abnormal result	Reference
Plasma lipids							
Wistar	50% of ad lib	11–22	13–16 w	F	Plasma triglyceride ↔, cholesterol ↔	No	Holemans *et al.* (1999)
Wistar	70% of ad lib	0–18	14 w	F	Plasma triglyceride↔	No	Ozaki *et al.* (2001)
Wistar	HF8%	0–22	26 w	M + F	Plasma HDL↓	Yes M	Lucas *et al.* (1996)
Wistar	IR	−7–22	112 w	M + F	Plasma triglyceride ↓	No M + F	Lewis *et al.* (2002)
Wistar	40% saturated fat	−7–22	3 w	C	Plasma triglyceride↑	Yes C	Guo & Jen (1995)
SD	24% saturated fat	−10–43	52 w	M + F	Plasma HDL↓	Yes M + F	Khan *et al.* (2003)
Wistar	24% saturated fat	−10–43	2 w	F	Plasma HDL↓	Yes	Koukkou *et al.* (1998)
Leptin							
Wistar	30% of ad lib	0–22	17 w	F	Plasma leptin↑, leptin receptor ↑ in pancreatic β cells	Yes	Vickers *et al.* (2001*b*)
Oxidative stress	50% of ad lib	1–22	16 w	M	*In situ* NADPH oxidase activity ↑	Yes	Franco *et al.* (2003)
Wistar	50% of ad lib	1–22	16 w	M	*In situ* NADPH oxidase activity ↑	Yes	Franco *et al.* (2003)
Adipose tissue							
Wistar	HF8%	0–43	6 w	M	Fat pad mass↓, adipocyte↓	No	Shepherd *et al.* (1997)
Wistar	30% of ad lib	0–22	25 w	F	Fat pad mass↑	Yes	Vickers *et al.* (2001*a*)
SD	24% saturated fat	−10–43	52 w	M + F	Bodyweight, fat pad mass↑	Yes	Khan *et al.* (2004)
Wistar	40% saturated fat	−7–22	3 w	C	Bodyweight, fat mass, fat% ↑	Yes C	Guo & Jen (1995)

Timing of protocol is relative to day of gestation (delivery is at 21–22 days in a rat); −10 indicates 10 days prior to mating and 43 days is equivalent to offspring post natal day 21 (weaning). Abbreviations: SD, Sprague-Dawley rat; HDL, high density lipoprotein cholesterol; IR, iron restricted diet (iron content 3 mg (kg diet)$^{-1}$); HF, Hope Farm low protein diet; w, postnatal week; ↔, no change; ↑, increased; ↓, decreased; M, male only; F, female only; C, combined male and female; M + F, both male and female considered individually.

offspring of severely (30% of *ad libitum* intake) calorie restricted rat dams.

Fat feeding. Guo & Jen (1995) report increases in bodyweight and fat composition (fat pad weight) in adult offspring of dams fed 40% fat during pregnancy and Khan *et al.* (2003, 2004) reported increased bodyweight and visceral fat deposits in 1-year-old offspring of dams fed 24% fat during pregnancy and suckling and reared on a normal diet but frank obesity in 1-year-old offspring of fat-fed dams fed a fat-rich diet throughout life (Khan *et al.* 2003). Plagemann *et al.* (1992) have ulilized a model of overfeeding (by reducing litter size and increasing milk availability during the suckling period) and shown that adult rats from small litters (3–4 pups) have increased bodywieght : length ratios than those from large litters (20–24). Vickers *et al.* (2001*b*) have shown hyperleptinaemia in offspring of calorie restricted rats fed a normal diet after birth, which is consistent with increased adipocyte mass. However, and this may be far more relevant to human metabolic syndrome and the predictive adaptive response hypothesis, offspring from protein restricted pregnancies are prone to development of obesity only when fed a hypercalorific postnatal diet. Thus, Ozanne *et al.* (2004) have observed increased body weight in offspring of protein restricted mice fed a cafeteria style diet (highly palatable, high carbohydrate) compared with those fed the control diet after weaning, and Vickers *et al.* (2001*a*) similarly showed increased weight in female offspring of calorie restricted rat dams reared on a 30% fat hypercalorific diet compared with controls fed the same diet after weaning.

In summary, offspring obesity is not reliably induced by maternal caloric, protein or iron restriction; however, maternal fat-feeding is associated with increased offspring weight and adiposity. The observation of normal body weight and adiposity in the restriction models in offspring fed a normal diet is consistent with the thrifty phenotype (Hales & Barker, 2001) and the predictive adaptive response (Gluckman & Hanson, 2004) hypotheses. The failure to develop adulthood obesity indicates that the adult diet is within the predictive adaptive range. Furthermore, and in keeping with these theories, there is limited evidence, from the few studies to have been attempted, to suggest that animals exposed to maternal low protein intake *in utero* followed by a hypercalorific diet in adulthood are frankly obese.

Therefore, in most studies of weight and adiposity, equivalence with the human metabolic syndrome remains to be adequately investigated.

Dyslipidaemia

Dyslipidaemia, typically increased triglyceride, increased low-density lipoprotein (LDL) and decreased high-density lipoprotein (HDL) concentrations in serum or plasma, is central to the diagnosis of the metabolic syndrome. Despite claims of analogy with the metabolic syndrome in many studies, few authors have evaluated serum lipid concentrations (Table 6). The rat is less than ideal as a model of human dyslipidaemia because of different plasma lipid profiles (carrying cholesterol mostly as HDL).

Caloric/protein/iron restriction. Plasma lipid profiles, either increased triglyceride or total cholesterol concentrations, are normal in offspring of two global restriction models (Holemans *et al.* 1999; Ozaki *et al.* 2001) whereas reductions in total cholesterol, HDL cholesterol and triglyceride concentrations are reported in male adult offspring of the 'Hope Farm' protein restriction model (Lucas *et al.* 1996). Female offspring of protein-restricted dams demonstrated a reduction in triglycerides only (Lucas *et al.* 1996). Male offspring of calorie restricted guinea-pig sows demonstrated increased plasma total cholesterol concentrations, compared with control offspring; however, female litter mates did not have altered plasma cholesterol concentrations compared with controls (Kind *et al.* 1999). Maternal iron restriction in rats does not appear to alter serum triglyceride or cholesterol concentrations. In 3-month-old male and female offspring of iron restricted Wistar rats, serum lipid concentrations were not found to be significantly different from controls (Lewis *et al.* 2001). Moreover, Lewis *et al.* (2002) reported lower serum triglycerides in 16-month-old male and female offspring of iron restricted dams. The restriction models in rats do not on the whole appear to induce lipid profiles in offspring with similarities to that of the human metabolic syndrome.

Fat feeding. In studies of fat feeding, authors have more frequently measured lipids and these models show consistent changes in offspring plasma lipid profiles. We (Ghosh *et al.* 2001; Khan *et al.* 2003) observed lowered HDL cholesterol and increased triglyceride concentrations in 160-day-old and 1-year-old male and female offspring of dams fed a lard-rich diet during pregnancy and suckling. Species differences may, however, exist and in this respect rabbits offer a better model of human disease as they are prone to the development of vascular lesions such as fatty streaks. Palinski *et al.* (2001) showed that offspring (combined male and female) of hypercholesterolaemic pregnant rabbits demonstrated unaltered plasma cholesterol but nonetheless serum lipid peroxides and fatty streak formation was reported. Using a model of altered litter size, Hahn (1984) has shown alteration of hepatic and adipose tissue enzyme activity such that rats from small litters (4 pups) were hypercholesterolaemic compared with offspring from large litters (14 pups). It is presumed that this model is a model of overfeeding as individuals in small litters have greater access to milk during the suckling period. As rats show unique plasma lipid profiles and in general do not demonstrate frank atherosclerosis, studies in rabbits or mice, particularly atherosclerosis prone knockout mice, could yield more relevant information. Given the wide selection of commercially available knockout mice, programming studies in susceptible strains, such as the apo-e knockout mouse, could provide a valuable method to elucidate the mechanisms involved in programming of adult phenotypes.

In summary, similarities with metabolic syndrome have infrequently been addressed in relation to dyslipidaemia, although, when studied, parallels with the human condition are apparent in some models.

Endothelial cell function

Although vascular endothelial cell dysfunction is not a current diagnostic criterion for the metabolic syndrome, there are calls for its inclusion (Bonora *et al.* 2003), and endothelial dysfunction is commonly associated with hypertension, hyperglycaemia and dyslipidaemia and contributes to the aetiology of cardiovascular disease (Heitzer *et al.* 2001). Indeed a primary role for reduced endothelial-dependent dilatation is the focus of one the current theories of insulin resistance (Caballero, 2003). A systemic pro-inflammatory status is now considered as a facet of the metabolic syndrome (Reilly & Rader, 2003) and is associated with vascular endothelial activation. This, in turn, has been linked to blunted vascular endothelium-dependent activation. There are numerous reports of abnormal endothelial cell function in offspring of dams subjected to different dietary interventions (Table 7).

Protein restriction. Protein restriction during development produces endothelial dysfunction in adult Wistar rat offspring, as determined by blunted endothelium-dependent vasodilator function in isolated resistance arteries (approximately 200 μm in diameter) mounted on a Mulvany-Halpern myograph (Brawley *et al.* 2003; Torrens *et al.* 2003). Endothelium-dependent ACh vasodilatation is mediated by a combination of nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarization factors (EDHF) that act directly to

Table 7. Offspring endothelial function in dietary models of developmental programming in the rat

Strain	Protocol	Intervention period	Age at measure (day of gestation)	Sex studied	Method of measure	Abnormality	Reference
Wistar	50% of ad lib	1–22	14 w	M + F	Aortic relaxation to ACh	Yes M + F	Franco *et al.* (2003)
SHR	50% of ad lib	1–22	13–16 w	M + F	Aortic relaxation to ACh	Yes M + F	Franco *et al.* (2002)
Wistar	50% of ad lib	11–22	14–17 w	F	Mesenteric relaxation to ACh	Yes	Holemans *et al.* (1999)
Wistar	70% of ad lib	0–18	28 w	M + F	Femoral relaxation to ACh	No M + F	Ozaki *et al.* (2001)
Wistar	SH9%	0–22	18 w	F	Aortic relaxation to ACh	No	Torrens *et al.* (2003)
Wistar	SH9%	0–22	18 w	F	Mesenteric relaxation to Isoprenaline	Yes	Torrens *et al.* (2003)
Wistar	SH9%	0–22	12, 23 w	M	Mesenteric relaxation to ACh	Yes	Brawley *et al.* (2003)
Wistar	24% saturated fat	−10–43	2 w	F	Femoral relaxation to ACh	Yes	Koukkou *et al.* (1998)
SD	24% saturated fat	−10–43	22 w	F	Femoral relaxation to ACh	Yes	Ghosh *et al.* (2001)
SD	24% saturated fat	−10–43	25 w	M + F	Mesenteric relaxation to ACh	Yes M + F	Khan *et al.* (2003)

Timing of protocol is relative to day of gestation (delivery is at 21–22 days in a rat); −10 indicates 10 days prior to mating and 43 days is equivalent to offspring post natal day 21 (weaning). Abbreviations: SHR, spontaneously hypertensive rat; SD, Sprague-Dawley rat; SH, Southampton low protein diet; w, postnatal week; M, male only; F female only; M + F, both male and female considered individually.

reduce vascular smooth muscle tone. Current data from the low protein feeding protocol indicate that only small resistance arteries are prone to endothelial dysfunction but that conduit arteries, such as aorta, are not affected in offspring of protein deprived dams (Brawley *et al.* 2003; Torrens *et al.* 2003). Lamireau *et al.* (2002) have also shown reduced endothelium-dependent vasodilatation of micro vessels (50 μm) from the cerebral circulation of offspring of protein restricted Wistar dams. These studies report increased smooth muscle sensitivity to nitric oxide, suggesting that compensatory mechanisms occur within vascular smooth muscle, presumably to maximize NO-induced dilatation. This may be indicative of a reduction in endothelium production, or bioavailability of NO, and such compensatory mechanisms may delay the onset of increased blood pressure, until their failure.

Caloric restriction. Adult (120 days old) female offspring of maternal Wistar rats which were fed 50% of the control dietary intake from mid to late gestation (when fetal growth is maximal) and through lactation demonstrated significantly reduced constrictor responses, reduced endothelium-dependent relaxation and enhanced sensitivity to the endothelium-independent dilator sodium nitroprusside, measured in small mesenteric arteries mounted on a Mulvany-Halpern myograph (Holemans *et al.* 1999). The difference in maximal contraction may reflect smaller vascular smooth-muscle mass in growth-retarded offspring. In a similar study (Wistar rats fed 50% of normal intake during pregnancy), increased blood pressure and decreased endothelial-derived relaxation in aortic rings were noted in male and female offspring (Franco *et al.* 2002*a*). Furthermore, mRNA expression of endothelial nitric oxide (eNOS) was lower in restricted males compared with control males at 14 weeks (Franco *et al.* 2002*a*) and superoxide synthesis (by assessment of NADPH oxidase activity) was increased in small mesenteric arteries due to activation of the angiotensin II type 1 receptor (Franco *et al.* 2003). The increase in superoxide generation, which to our knowledge has not been investigated in any other model, could contribute to reduced endothelium-dependent dilatation through reduction of bioavailable NO or tissue oxidative damage. Moreover, mitochondrial dysfunction, a possible consequence of maternal dietary restriction, has been implicated, together with oxidative stress, as a contributory factor in the development of atherosclerosis (Ballinger *et al.* 2002). In contrast to other reports, Ozaki *et al.* (2000) described male offspring of globally restricted dams (30% of *ad libitum* intake) as having modest vascular abnormalities (increased maximal thromboxane mimetic induced contraction and enhanced sensitivity to potassium) at 200 days but no alteration to endothelium-dependent vasodilatation. Taken together these studies suggest that the severity of the offspring endothelial phenotype in rats is relative to the degree of maternal caloric restriction.

Fat feeding. Maternal diets rich in animal fat also produce endothelial dysfunction in offspring, evidenced by reduced endothelium-dependent dilatation in femoral and mesenteric resistance vessels (Ghosh *et al.* 2001; Khan *et al.* 2003), and also in aorta (Armitage *et al.* 2004), from both male and female offspring. In these studies there was no change in smooth muscle sensitivity to nitric oxide, but preliminary evidence from Affymetrix gene array analysis showed eNOS expression to be lower in aorta of 1-year-old offspring from lard exposed dams compared with controls

(Armitage *et al.* 2004). Mechanisms underlying the blunted endothelium-dependent dilatation may, however, be vascular bed specific. The mechanism underlying endothelial dysfunction in mesenteric arteries of offspring exposed to a maternal fat- rich diet has been further characterized by our group and we have reported that blunted endothelium-dependent relaxation is attributable to a reduction in the EDHF component of relaxation in this vascular bed (Taylor *et al.* 2004). Khan *et al.* (2004) have recently found evidence for the predictive adaptive response hypothesis in this model. Adult rats maintained on the same fat-rich diet that they were exposed to *in utero* (continued fat-feeding) demonstrated improved endothelium-dependent vasodilatation compared with animals adapted to a fat-rich diet *in utero* and subsequently reared on a control diet in adulthood (Khan *et al.* 2004).

Bio-markers of endothelial cell activation, although frequently described in the metabolic syndrome, have not been studied in offspring from models of maternal nutritional imbalance. This is partly a reflection of the resistance of the rat endothelium to activation stimuli. Increased superoxide generation (NADPH oxidase activity) and decreased antioxidant defence (decreased superoxide dismutase activity) in offspring of calorie restricted rats is a probable stimulus to endothelial cell activation (Franco *et al.* 2002*c*) and in other species, endothelial cell activation would be expected. It may be relevant that tissues from adult female offspring of protein-malnourished (8%) dams show some evidence for increased sensitivity to inflammatory stimuli; Merezak *et al.* (2004) have demonstrated enhanced islet cell susceptibility to a combination of the cytokines IL-1β, TNF-α and IFN-γ in 3-month-old offspring. Should this also occur at the level of the endothelial cell, endothelial cell activation could be anticipated.

In summary, endothelium-dependent relaxation is an accompaniment to most of the nutritional developmental programming models in rodents. Given the pivotal role proposed for reduced endothelium-dependent dilatation in insulin resistance and type 2 diabetes (Meigs *et al.* 2004), these observations coupled with the evidence for insulin resistance undoubtedly have significant parallels with the human metabolic syndrome. These studies, however, require expansion to investigations of the inflammatory response that might be best attempted in murine models.

Disparity amongst diets

One of the major issues in this rapidly developing field, and one which often hinders firm conclusions being drawn, lies in the disparity between diets used. The ideal composition of diets that are fat supplemented or protein restricted always presents understandable difficulty. Any experimental alteration of one component by necessity leads to alteration of another, and problems arise in establishing compositional balance. This is best exemplified by comparison of the different protein restriction diets, as these have been most frequently employed.

Table 2 serves to show the many differences between laboratories in the low protein diets used, and offers some explanation for the phenotypic variance. The 'Southampton' and 'Hope Farm' diets differ principally in carbohydrate content, fat content and fatty acid profile. The 'Southampton' diet provides twice as much fat (corn oil) as the 'Hope Farm' diet (soybean oil). Both are rich in the omega-6 polyunsaturated fatty acid (PUFA) linoleic acid and monounsaturated oleic acid, accounting for almost 80% of the fatty acids. However, the 'Hope Farm' diet, containing soybean oil, also contains three times more of the essential omega-3 PUFA α-linolenic acid than the 'Southampton' diet. As previously discussed, a potential programming effect by the reduction in cardio-protective omega-3 fatty acids in any diet should be considered. Certainly, omega-3 deprivation has been shown to alter endothelial function, insulin sensitivity and development (Carlson & Salem, 1991; Storlien *et al.* 1991; Connor, 1997; Brown & Hu, 2001) and produces longstanding programming of hypertension when deprivation occurs early in life (Weisinger *et al.* 2001; Armitage *et al.* 2003). Dietary formulations should bear this caveat in mind and ensure adequate supply, for example the diet formulated by Athauda *et al.* (2004) that contains soy oil which is both omega-3 and omega-6 PUFA rich. The observation of disparity in dietary composition is not new, having being highlighted by Langley-Evans (2000), but the potential importance of this variation between diets has attracted little attention. The 'Southampton' diet is not the only omega-3 PUFA deficient diet; there are other diets that may inadvertently induce omega-3 essential PUFA deficiency (Galler & Tonkiss, 1991; Woods *et al.* 2001). Tissue (brain, liver) omega-3 PUFA levels are reduced in 32- to 36-day-old offspring of dams fed the 'Southampton' low protein diet during pregnancy (Burdge *et al.* 2003) as well as in 6-month-old offspring of dams fed a lard-rich diet in pregnancy and suckling (Ghosh *et al.* 2001). Thus, two different maternal dietary manipulations appear to have a common outcome, and it is possible that programming of altered membrane fatty acid profiles might give rise to altered membrane protein function, and therefore altered physiology.

The carbohydrate content of the 'Southampton' diet is predominantly starch and of the 'Hope Farm' diet predominantly glucose. The 'Hope Farm' diet, with 68% glucose (w/w), represents a high glucose load. The phenotype of insulin resistance and pancreatic dysfunction could therefore be induced by the high glycaemic index of the 'Hope Farm' diet rather in addition to the effects of protein restriction. Maternal and feto-placental

plasma glucose is increased in the 'Hope Farm' protocol (Fernandez-Twinn *et al.* 2003) and there is considerable experimental evidence from studies to suggest that maternal diabetes in rats programmes insulin resistance in offspring (reviewed by Van Assche *et al.* 2001).

Compared with all other low protein diets reviewed, the 'Southampton' diet contains significantly more methionine, which is added to the diet in order to avoid sulphur deficiency when casein is the sole protein source. This leads to an excess of methionine, relative to total protein intake and that of other individual amino acids, in the protein deficient diets. High concentrations of circulating methionine may lead to hyperhomocysteinaemia, a recognized cardiovascular risk factor that impairs endothelial function. It is not clear whether high fetal homocysteine levels may promote the later development of cardiovascular disease, but maternal serum levels of homocysteine are raised after only 4 days of feeding the 'Southampton' diet (Petrie *et al.* 2002) and are associated with altered gene methylation status in liver tissue of fetuses from protein restricted dams (Rees *et al.* 2000). As gene methylation alters gene transcription, developmentally induced perturbation of gene methylation may lead to inappropriate transcription, and therefore expression, of gene products during development, leading to programmed alteration of the adult phenotype. Indeed, Weaver *et al.* (2004) have recently shown that aberrant gene methylation of the hippocampal glucocorticoid receptor underlies the programming of altered adult stress reactivity in rats of dams with poor nursing habits (grooming, licking and arch backed nursing). Also, adult offspring that were growth restricted *in utero* (following uterine artery occlusion) have been found to have reduced DNA methylation of key genes involved in the apoptosis pathway, in kidney tissue (Pham *et al.* 2003). CpG hypomethylation of the p53 gene, involved in the apoptosis pathway, was accompanied by increased expression of p53 mRNA, decreased mRNA expression of the anti-apoptotic protein Bcl-2 (Pham *et al.* 2003) and increased nephron apoptosis. Recent data suggest that maternal hyperhomocysteinaemia is peculiar to the 'Southampton' diet and is not elevated in either 'Hope Farm' or lard-rich dietary protocols at 4 days gestation (J. McConnell & S. Ozanne, personal communication). There is no report of plasma hyperhomocysteinaemia in caloric restriction or in IUGR offspring from uterine artery ligated dams. Supplementing the 'Southampton' diet with glycine (which, as well as folate supplementation, achieves reduction of plasma homocysteine) results in a normalization of blood pressure, suggesting that the methionine load contributes to the 'Southampton' phenotype (Jackson *et al.* 2002). Additionally, glycine supplementation reverses the maternal resistance artery endothelial dysfunction seen in this dietary model (Brawley *et al.* 2004), further evidence that hyperhomocysteinaemia may play a role in the programming stimulus of the 'Southampton' diet.

Most studies report that following the requisite period of dietary manipulation, animals are fed a standard rat chow. Some commercially available diets incorporate soy protein as the protein source and therefore contain relatively high levels of isoflavones (Degen *et al.* 2002; Kato *et al.* 2004). The isoflavones of interest in soy protein are the phytooestrogens genestein and diadzein, both oestrogenic compounds (Lissin & Cooke, 2000). These phytooestrogens may have beneficial actions upon the cardiovascular system – including promotion of vessel dilatation, antioxidant defences, and reduction of endothelial cell inflammatory markers (Lissin & Cooke, 2000). It is feasible that a phytooestrogen-containing 'control chow' fed in adulthood will lessen the magnitude of developmentally programmed alterations to cardiovascular function. As opposed to the detailed description of maternal control and experimental diets, the protein source of chow fed to adult offspring is not generally provided in publications, but this should routinely be reported in future work.

Sodium content is another dietary parameter to be considered. There is evidence within the literature to support a role for increased (Di Nicolantonio *et al.* 1990; Contreras & Oparil, 1992; Contreras, 1993; Contreras *et al.* 2000) and decreased maternal sodium intake as a stimulus to the developmental programming of offspring blood pressure and cardiovascular disease. Studies have focused on sodium loading and effects on the offspring renin–angiotensin system and the aldosterone axis and there are no reports of the effect of maternal sodium loading upon characteristics of metabolic syndrome in offspring. Notwithstanding, there is evidence that offspring of sodium loaded (Di Nicolantonio *et al.* 1990) and sodium deprived (Roy-Clavel *et al.* 1999; Battista *et al.* 2002) dams show reduced birthweight and altered growth trajectories and that offspring of sodium loaded dams demonstrate increased appetite for a palatable cafeteria style diet (Contreras, 1993) and increased sodium appetite (Contreras & Kosten, 1983; Contreras & Ryan, 1990) that is not associated with altered peripheral taste sensation (Bird & Contreras, 1987). It is therefore possible that inappropriate maternal sodium intake (either high or low) can programme a phenotype that displays adult risk factors for the development of the metabolic syndrome. It is likely, however, that any programmed effects of maternal sodium manipulation are of a different origin from those produced by exposure to a maternal low-protein diet. Langley-Evans & Jackson (1996) demonstrated that sodium loading offspring of protein deprived dams did not result in further increases in blood pressure, suggesting that those animals did not have a shifted pressure–natriuresis curve. Further study of facets of the metabolic syndrome in offspring of

sodium manipulated dams would be of interest. For the present, however, sodium content of experimental diets is one issue that should be controlled for in diets produced 'in-house'.

Conclusion

The variation in feeding regimens, dietary challenges and, importantly, the techniques used to assess outcome measures undoubtedly contribute to some of the uncertainty and inconsistencies observed across developmental programming models. However, sound evidence supports the suggestion that elements of the metabolic syndrome may be induced by a wide variety of nutritional imbalances in maternal diet. Although there is much variation in both the inherent severity of dietary challenge, and the form of challenge, the various animal models seem to produce a converging phenotype.

It may reasonably be concluded that abnormal insulin/glucose homeostasis is programmed by *in utero* dietary insult. Data are more variable with regard to the programming of blood pressure, exposure to the 'Hope Farm' low protein diet does not result in offspring blood pressure changes. There are data to support dyslipidaemia, but the rat is not the ideal model in which to assess this parameter and murine studies would be more informative. There is a paucity of data relating lean body mass or fat pad weight to nutritional insult, but in terms of bodyweight data, it does not appear that obesity is reliably programmed by maternal diet, although animals have seldom been challenged with high calorie diets. Impaired vascular endothelial cell dilatation is programmed by all dietary protocols, and appears to be one of the most robust phenotypes observed in offspring of diet challenged pregnancies. Given the accepted association of reduced endothelium-dependent dilatation within insulin resistance and type 2 diabetes in man and the proposal for an aetiological role, the prevalence of this defect in the different models suggests an important determinant in evolution of the offspring phenotypes reported. There is a shortage of data with regard to inflammation, leptin resistance and oxidative status and these factors represent an attractive focus for further research. This review highlights the variety of end-point measures reported throughout the literature and, reflecting current knowledge, these cannot throw much light on the fundamental underlying mechanisms at the gene and cellular level. To date, maternal dietary imbalance models have highlighted the harmful consequences for offspring. One of the natural extensions of this body of research is to search for intervention strategies in order to offer benefit for humans. In particular, the effect and range of predictive adaptive responses requires further examination. Most models appear to show predictive adaptive responses and the modification of programming sequelae by postnatal and adult diet is highly relevant to humans where, in the main, postnatal nutrition is plentiful. Further characterization of the range (and limitation) of predictive adaptive responses is required in all models, and if successful will provide useful data. The focus of this review has deliberately been placed upon the relationship between maternal nutritional interventions and offspring phenotype, and proposed mechanistic pathways have deliberately not been discussed. Offspring phenotypic characteristics described akin to those of the metabolic syndrome are also induced by antenatal administration of glucocorticoids (Seckl *et al.* 2000; Dodic *et al.* 2002; O'Regan *et al.* 2003), and interventional studies imply a role for activation of the maternal HPA axis in some of the models described. However, this is not a proven stimulus in all models. Other pathways, e.g. maternal hyperglycaemia, mitochondrial dysfunction and oxidant stress, are also implicated. The challenge for the design of future studies is to combine *in vivo* and *in vitro* approaches, whole animal systems physiology and signal transduction and gene expression studies. Only by doing so will it be possible to reveal the mechanisms by which a variety of different exposures, including macronutrient imbalance, result in a wide range of outcomes that collectively demonstrate the characteristics of the metabolic syndrome. Current strategies seem to focus on baseline values for parameters, and one future direction could be the study of stimulated or challenged responses; however, a detailed understanding of baseline function is vital before this approach can be taken. Maternal dietary imbalance in animals appears, on the whole, to produce features of the metabolic syndrome. By careful selection of species, diet and measurement techniques, the component models can be used in longitudinal studies to examine the aetiology of and the interactions between the spectrum of disorders that collectively define the metabolic syndrome in man.

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